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Published in:
New Phytologist

DOI:
10.1111/nph.17190

Publication date:
2021

New Phytologist

Research

The maximum growth rate hypothesis is correct for eukaryotic photosynthetic organisms, but not cyanobacteria

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Received: *22 October 2020*
Accepted: *23 December 2020*

New Phytologist (2021)
doi: 10.1111/nph.17190

Key words: growth rate hypothesis, maximum growth rate, phospholipids, phosphorus productivity, polyphosphate, RNA, rRNA operon copy number.

Summary

- The (maximum) growth rate (μ_{max}) hypothesis predicts that cellular and tissue phosphorus (P) concentrations should increase with increasing growth rate, and RNA should also increase as most of the P is required to make ribosomes.
- Using published data, we show that though there is a strong positive relationship between the μ_{max} of all photosynthetic organisms and their P content (% dry weight), leading to a relatively constant P productivity, the relationship with RNA content is more complex.
- In eukaryotes there is a strong positive relationship between μ_{max} and RNA content expressed as % dry weight, and RNA constitutes a relatively constant 25% of total P. In prokaryotes the rRNA operon copy number is the important determinant of the amount of RNA present in the cell. The amount of phospholipid expressed as % dry weight increases with increasing μ_{max} in microalgae. The relative proportions of each of the five major P-containing constituents is remarkably constant, except that the proportion of RNA is greater and phospholipids smaller in prokaryotic than eukaryotic photosynthetic organisms. The effect of temperature differences between studies was minor.
- The evidence for and against P-containing constituents other than RNA being involved with ribosome synthesis and functioning is discussed.

Introduction

The growth rate hypothesis (Elser *et al.*, 1996, 2000; Sterner & Elser, 2002) states that rapid growth requires increased numbers of ribosomes for protein synthesis, which in turn means that cellular concentrations of phosphorus (P)-rich RNA (largely ribosomal RNA) will also increase. Consequently the hypothesis predicts that RNA content and, consequently, organism P concentrations should increase with increasing growth rate (Elser *et al.*, 1996; Main *et al.*, 1997; Sterner & Elser, 2002; Moreno & Martiny, 2018). It should be noted that the growth rate hypothesis originated with differences in P content between slow-growing freshwater copepods and rapidly growing cladocerans, mainly *Daphnia*, being almost entirely explained by differences in RNA content (Sterner, 1995; Elser *et al.*, 1996; Sterner & Elser, 2002). In other words, changes in P content are dominated by changes in RNA content (mainly rRNA, but also mRNA and tRNA, which should also increase with growth rate) and, all else being equal, the relative proportions of P-containing compounds do not remain constant with growth rate. However, Elser *et al.* (2003) provide evidence that the amount of P in RNA constitutes 49% of the total P in a number of prokaryotic and eukaryotic heterotrophs. Most of the information relating to the growth rate hypothesis is for an individual species growing under various forms of resource limitation. The few interspecific studies of the growth rate hypothesis either do not include any photosynthetic organisms (Sutcliffe, 1970; Elser *et al.*, 2003), or concentrate only on N : P ratios in higher plant leaves (Reich *et al.*, 2010) or N : P ratios in phytoplankton (Flynn *et al.*, 2010), though the latter does include fluorescence data for RNA (see 'RNA' in Materials and Methods).

Here we address the relationship, at the interspecific level, between μ_{max} (a cardinal characteristic of any organism (Flynn & Skibinski, 2020)) of photosynthetic organisms and their nitrogen (N) and P contents, P productivities (g dry matter g^{-1} phosphorus d^{-1}) and P-containing constituents, in both prokaryotes and eukaryotes. We reverse the logic of the growth rate hypothesis by starting with the strong positive relationship between μ_{max} and P content and then addressing the reason for this. RNA content is a part of the answer, and we discuss the possibility that the other P-containing constituents are involved directly or indirectly in ribosome synthesis and function.

Materials and Methods

Data

We searched (from Google to Web of Science) the literature (a total of 79 publications) for data on maximum growth rate

(μ_{max}), organism N and P content and P-containing cellular constituents (polyphosphate, RNA, DNA, phospholipids and phosphate esters and anhydrides) expressed as a percentage of dry weight and/or total P in photosynthetic organisms. Where the relationships between μ_{max} and N and P content are compared, only data for all three parameters for a given species that were obtained from the same paper (or in two instances from the same group) were used. Where comparisons are made between μ_{max} and a constituent (e.g. RNA) only data for both parameters given in the same paper were used. The number of observations is given in the legend of each table and figure.

Maximum growth rate

Only μ_{max} is considered here and it is assumed that growth was balanced (i.e. all cellular constituents increase at the same rate) and that local conditions allowed for sufficient resources, which would include temperature and light for the latter, when the measurements were made. Only μ_{max} involving measurements of the increase in cell number or some measure of biomass (e.g. fresh weight, dry weight) over time were used. All μ_{max} values are expressed as specific growth rate with units of d^{-1}. Data provided as doublings d^{-1} (base 2) were converted to μ_{max} by multiplying by $\log_e 2$ (= 0.6931). Where there was more than one reported value for μ_{max} of a species, the highest value was used as it is assumed that this represents the true (or truer) μ_{max}. The assumption of balanced growth allows μ_{max} measured as, for example, increases in cell density to be expressed as g dry weight g^{-1} dry weight d^{-1}. Values for μ_{max} were not corrected for temperature, because though there are appropriate Q_{10} values for growth rate, there are no comparable values for N, P or RNA content of organisms (see Moreno & Martiny (2018) for a full discussion).

Organismal N and P

Values for cell or tissue N and P are for organisms growing at μ_{max}. Values for N and P were obtained from the same paper as μ_{max} and were used only where these values were given as % dry weight or where it is possible to calculate dry weight. All these data were used to calculate P productivities, and extra data were added from other sources where only μ_{max} and P content were provided. The benefits of using dry weight as a standard measure for biomass are outlined elsewhere (Rees, 2014; Raven, 2015) under 'What is the effect of temperature?' in the Discussion.

Storage of N or P (as polyphosphate or phosphate) can, both in principle and in practice, have a marked effect on values for organism N or P. This is more likely to occur when an organism is grown with excess external sources of N or P to prevent resource limitation and to ensure μ_{max} and is why Raven (2013a) explicitly deducts polyphosphate from an inventory of major P-containing fractions. The main reason for including polyphosphate is that it may have an important role in biosynthesis and not simply as a form of P storage (the non-storage role of polyphosphate is discussed under 'Polyphosphate/phosphate/phytate').

Phosphorus productivity

Phosphorus productivity (g dry weight g^{-1} phosphorus d^{-1}) was calculated as:

$$\frac{\mu_{max}}{P}$$

where μ_{max} is maximum growth rate (d^{-1}) and P is the proportion of dry weight that is phosphorus (g phosphorus g^{-1} dry weight).

A single, exceptionally high, P productivity (536 g dry biomass g^{-1} P d^{-1}) for *Ulva rigida* (Lavery & McComb, 1991) is not included. This high rate is largely due to a very low tissue phosphorus content of 0.04%, which is the lowest for any photosynthetic organism recorded here. *Chaetomorpha linum* from the same site had a P productivity of 119 g dry biomass g^{-1} P d^{-1} and a tissue phosphorus content of 0.2% (Lavery & McComb, 1991), suggesting that the high P productivity for *U. rigida* is not due to any property of the site that it was collected from.

RNA

Values for cell or tissue RNA are for organisms growing at μ_{max}. There are a variety of potential problems associated with data for RNA and protein content, most of which have been addressed elsewhere (Flynn *et al.*, 2010). Raven (2013a) has highlighted some of the problems associated with the extraction of protein and, to a greater extent, RNA, and there are additional problems relating to the measurement of RNA (and DNA) with fluorescent probes (Mordy & Carlson, 1991; Hildago *et al.*, 2017). There may be instances where these problems do not occur or have been prevented, but there are examples where the use of fluorescent probes for both DNA and RNA give very low values for these nucleic acids (mainly microalgae and a few macroalgae), and these data are not included here. This is not to suggest that values obtained with fluorescent probes are incorrect. Rather, for internal consistency and because there are more published values, RNA values reported here used the orcinol method mainly, but also the UV method (Herbert *et al.*, 1971; Geider & LaRoche, 2002) and are expressed as % total dry weight and/or %P. Where relevant it is assumed that 9.1% of RNA is P (Sterner & Elser, 2002).

Phospholipids

Values for phospholipids are for organisms growing at μ_{max}. Where relevant it is assumed that 4.2% of phospholipids is P (Sterner & Elser, 2002). There are conflicting values for the amount of phospholipid in the Haptophyta. Values for phospholipids in *Isochrysis galbana* range from 0.12% (Cañavate *et al.*, 2017) to 5.2% (Zhu *et al.*, 1997) and 5.5% of total dry weight (Fidalgo *et al.*, 1998). For *Diacronema vlkianum* phospholipids are 1.5% of total lipid and 0.24% of dry weight; total lipid is 15.9% of dry weight (Cañavate *et al.*, 2017). Other values for *D. vlkianum* are similar: phospholipids are 3.3% of total lipid (Armada *et al.*, 2013, albeit the same group as Cañavate *et al.*,

2017) and total lipid is 17.9% of total dry weight (Fradique *et al.*, 2013). Phospholipids are a minor component of lipid in *Pavlova lutheri* (Eichenberger & Gribi, 1997). In *Tisochysis lutea* phospholipids in the light are either the most abundant (Lacour *et al.*, 2012) or the second most abundant lipid class (after glycolipids) (Marchetti *et al.*, 2018). Phospholipids make up *c.* 33% of the total intact polar lipid during P-replete growth in *Emiliania huxleyi* (Shemi *et al.*, 2016). There is a clear discrepancy in the apparent content of phospholipids in the Haptophyta that needs resolution. Where there were three values (*I. galbana* and *Nannochloropsis gaditana*), two of the three values for phospholipids were in close agreement and the one with the greater μ_{max} was used. For other values there was only one published value for μ_{max} and phospholipid content.

Other P constituents

Values for the other P constituents are for organisms growing at μ_{max}. Values for DNA, polyphosphate and P-esters and anhydrides were obtained from the literature and either expressed as % total dry weight and/or %P.

Statistics

Reduced major axis (RMA) regression (Sokal & Rohlf, 1995) was used to describe relationships between μ_{max} and cellular constituents. For these analyses the line-fitting package SMATR v.2.0 (Warton *et al.*, 2006; http://www.bio.mq.edu.au/ecology/SMATR/) was used. Differences between phospholipid content and logged values for RNA : phospholipids ratio in prokaryotic and eukaryotic photosynthetic organisms were investigated using *t*-tests. Differences between DNA content as % of dry weight in microalgae and macroalgae and an angiosperm were investigated using a Mann–Whitney rank sum test. The effect of growth temperature and μ_{max} on %P and RNA content were determined using multiple regressions. All statistical tests were performed in SIGMAPLOT v.14.

Results

Relationships between maximum growth rate and N and P content

There was a strong positive linear relationship ($r^2 = 0.60$) between μ_{max} and P content expressed as percentage of organism dry weight (Fig. 1a). By contrast, there was a much weaker relationship ($r^2 = -0.35$) between μ_{max} and nitrogen content expressed in terms of percentage of organism dry weight (Fig. 1b), and the relationship was stronger ($r^2 = 0.45$) if the data were fitted to a rectangular hyperbola ($n = 58$ for both Fig. 1a and b). Using a more extensive collection of data ($n = 78$), for μ_{max} and P content expressed as percentage of organism dry weight, the relationship was stronger

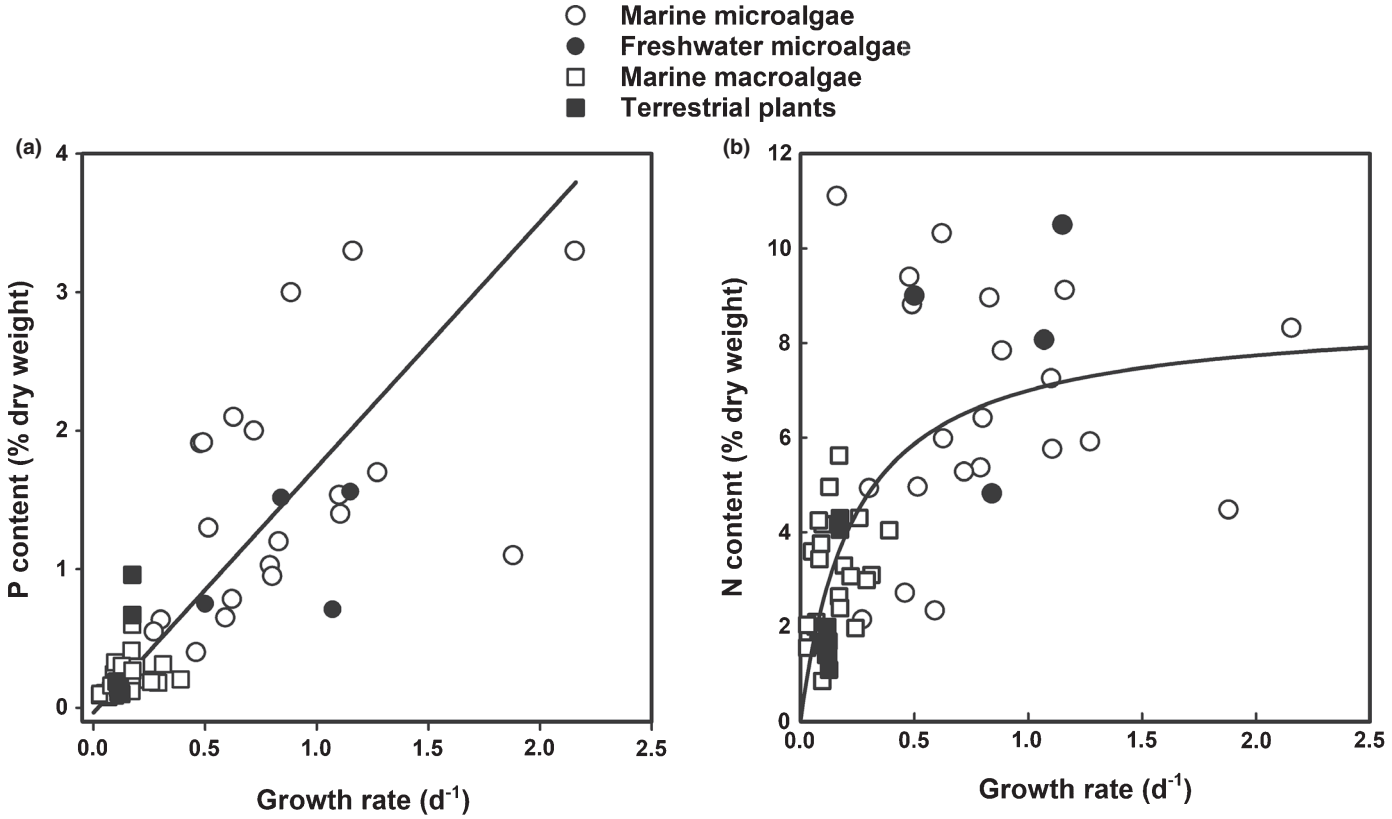

Fig. 1 Relationship between maximum growth rate (d^{-1}) and (a) phosphorus (P) (% dry weight) and (b) nitrogen (N) content (% dry weight) of photosynthetic organisms ($n = 58$). The reduced major axis regression equation and coefficient of determination for the relationship between maximum growth rate and phosphorus content are as follows: $y = -0.04 + 1.78x$; $r^2 = 0.60$, P (slope = 0) < 0.001. The fitted rectangular hyperbola shows the relationship between maximum growth rate and nitrogen content ($r^2 = 0.45$).

Table 1 Mean (±SE) and median phosphorus productivities (g dry biomass g^{-1} P d^{-1}) for different groups of photosynthetic organisms growing at maximum growth rate.

	P productivity (g dry biomass g^{-1} P d^{-1})		
	Mean	Median	*n*
Marine microalgae	77 ± 8	72	30
Freshwater microalgae	75 ± 9	67	9
Marine macroalgae	72 ± 10	58	24
Terrestrial plants	71 ± 9	77	15
All photosynthetic organisms	74 ± 5	66	78
Cyanobacteria	74 ± 4	74	7

Cyanobacteria include marine and freshwater species.

($r^2 = 0.67$). There was a minor and nonsignificant ($P = 0.336$) effect of temperature on P content (%P = 0.44 + (1.35 × μ_{max}) − (0.016 × temp), $r^2 = 0.67$). There were no differences between the P productivities of marine and freshwater microalgae, marine macroalgae and terrestrial plants (angiosperms and a fern) ($n = 78$) (Table 1).

Distribution of the five major categories of P-containing constituents as a percentage of total P

The distribution of the five major categories of P-containing constituents as a percentage of total P within photosynthetic organisms is shown in Table 2. The main constituents were polyphosphate (and phosphate) and RNA. If it is assumed that the sole function of polyphosphate + phosphate is phosphate storage, the relative amounts of P in the other four categories are very similar to those reported by Raven (2013a).

There was a significant difference in the phospholipid content (as a proportion of total P) between prokaryotic and eukaryotic photosynthetic organisms ($t = -2.432$; df = 21; $P = 0.024$), being five times greater in eukaryotic photosynthetic organisms (Table 2). There were insufficient data to make the comparison based on dry weight. There was an even greater difference between prokaryotic and eukaryotic photosynthetic organisms ($t = 6.936$; df = 10; $P = <0.001$) in the RNA : phospholipids ratio (Table 2).

Relationships between maximum growth rate and RNA and phospholipids in eukaryotic photosynthetic organisms

There were no relationships between any of the five major categories of P-containing compounds expressed as a proportion of dry weight and μ_{max} except for RNA and, possibly, phospholipids. For the other categories there were insufficient data (polyphosphate, $n = 1$; phosphate esters, $n = 0$) or no relationship (DNA, $n = 7$). However, there was a significant difference (Mann–Whitney $U = 7$; $P = 0.002$) between the DNA content (as % dry weight) of faster growing microalgae (marine and freshwater; 0.54%) and that of macrophytes (macroalgae and a terrestrial angiosperm; 0.28%).

The slope of the relationship for eukaryote phospholipids and μ_{max} was not significantly different from zero, but the relationship was positive ($r^2 = 0.28$). If a high value for phospholipid content of the freshwater diatom *Stephanodiscus minutulus*, which has a higher lipid content than protein even at μ_{max} (Lynn *et al.*, 2000) is removed, the slope of the positive relationship was significantly different from zero ($r^2 = 0.42$; Fig. 2). A comparison of the slope and intercept of this relationship with that for Fig. 1(a), and assuming that 4.2% of phospholipids is P (Sterner & Elser, 2002), suggests that phospholipids constitutes a roughly constant 11% of total P in photosynthetic organisms, which is very similar to the value for eukaryotic phospholipids (which includes the value for *S. minutulus*) in Table 2.

Table 2 Percentage of the major phosphorus-containing fractions in photosynthetic organisms growing at maximum growth rate as mean values (± SE).

	(% total P) Mean ± SE	*n*
DNA	9 ± 2	18
P-esters	10 ± 3	5
Cyanobacteria		
RNA	46 ± 6	4
Phospholipids	3 ± 1	4
RNA : phospholipids	20 ± 5	4
Polyphosphate	29 ± 9	6
Eukaryotes		
RNA	25 ± 3	18
Phospholipids	14 ± 2	19
RNA : phospholipids	2 ± 0.4	8
Polyphosphate/phosphate	35 ± 8	7

Values for DNA and P-esters are for all photosynthetic organisms; RNA, phospholipids and polyphosphate are given as separate values for cyanobacteria and eukaryotes. It should be noted that phosphate rather than polyphosphate makes a major contribution in angiosperms, and one value (Bieleski, 1968) is included here, but only two published values (Robson *et al.*, 1959; Bieleski, 1968) distinguish between DNA and RNA.

There was a strong positive relationship ($r^2 = 0.66$) between μ_{max} and RNA as a percentage of dry weight in eukaryotic photosynthetic organisms (marine and freshwater microalgae and terrestrial plants) (Fig. 3). There was a nonsignificant ($P = 0.226$) effect of temperature on RNA content (RNA = 4.1 + (3.49 × μ_{max}) − (0.118 × temp), $r^2 = 0.71$). A comparison of the slope and intercept of this relationship with that for Fig. 1(a), assuming that 9.1% of RNA is P (Sterner & Elser, 2002), suggests that RNA constitutes a roughly constant 25% of total P in eukaryotic photosynthetic organisms, which is very similar to the value for eukaryotic RNA in Table 2.

Relationships between maximum growth rate and RNA in cyanobacteria: the importance of the rRNA operon copy number

For a given μ_{max}, cyanobacteria generally had a greater RNA content (Fig. 3), despite identical P productivities (Table 1). For heterotrophic prokaryotes growing at < 2.0 d^{-1}, there was a similar relationship to that of cyanobacteria (Fig. 3). Though most prokaryotes had a greater RNA content for a given μ_{max} than eukaryotes, there were two exceptions with lower RNA contents

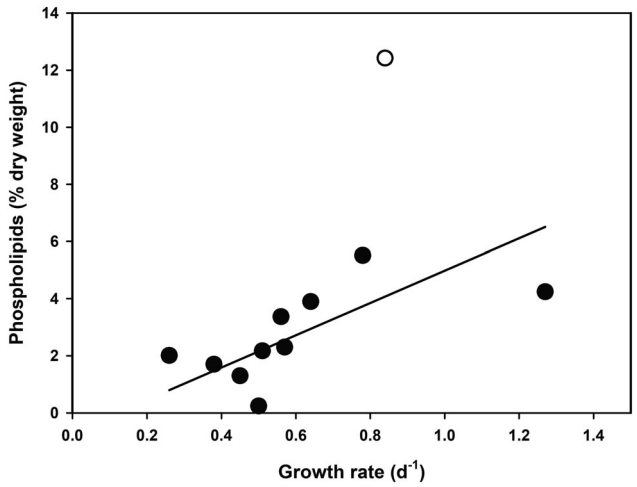

Fig. 2 Relationship between maximum growth rate (d^{-1}) and phospholipid content (% dry weight) of eukaryotic microalgae. The reduced major axis regression equation and coefficient of determination for the relationship between maximum growth rate and phosphorus content are as follows: $y = -0.68 + 5.66x$; $r^2 = 0.42$, P (slope = 0) = 0.043, $n = 10$. The open circle represents data for the freshwater diatom *Stephanodiscus minutulus* (Lynn *et al*., 2000), but it is not included in the regression analysis. Data were obtained from Fidalgo *et al*. (1998), Pahl *et al*. (2010) and Cañavate *et al*. (2017).

that were comparable to eukaryotic photosynthetic organisms. The two exceptions were *Prochlorococcus marinus* subsp. *pastoris* str. CMP1986 (also known as Med4) and *Mycobacterium bovis*, that both have a single copy of the rRNA operon (Cox, 2004; Schirrmeister *et al*., 2012). By contrast, the other bacteria have two to six copies (Cox, 2004; Schirrmeister *et al*., 2012); there is no value for *Nostoc paludosum*, but other species and strains of *Nostoc* possess three to six copies (The Ribosomal RNA Database; https://rrndb.umms.med.umich.edu/search/).

Discussion

Given the strong relationship between μ_{max} and P content, what is (are) the most likely P-containing constituent(s) that is (are) required for growth, and why is more P required for rapid growth? One possibility is that most of the P is allocated to RNA (as stated by the growth rate hypothesis). However, RNA accounts for only 25% (in eukaryotic photosynthetic organisms) or 50% (in prokaryotic photosynthetic organisms) of total P. Clearly this leaves 50 to 75% of total P unaccounted for. Either the growth rate hypothesis is only a partial explanation of the relationship between μ_{max} and P content, or the other four categories of P-containing constituents are directly or indirectly involved with RNA synthesis and/or ribosome functioning. This latter possibility, which would leave the growth rate hypothesis largely intact, is explored under 'Role of P-constituents other than RNA'. However, we start by addressing two important questions relating to the data.

Does μ_{max} represent the true maximum growth rate?

The answer to this question is that it is impossible for anyone to be certain that their measurement of μ_{max} is a measure of the true maximum growth rate. Fenchel (1974) refers to his maximum growth rate (r_m) as an 'approximation'. Given that we restricted our μ_{max} values to those papers that also contained information on N, P, RNA and phospholipids, then our μ_{max} is possibly even more of an approximation. However, it could be argued that as our techniques improve (there is independent evidence that they do not) our 'approximate' values should become closer to the true values. Within the context of our data, we would also expect, if the measured μ_{max} increased in the future, that the organism's P content would also increase.

What is the effect of temperature?

The effect of temperature on P productivity was negligible across all photosynthetic organisms and minor with RNA content in eukaryotes. There is evidence for an increase in the amount of cellular or tissue RNA with a decrease in temperature in photosynthetic organisms (Woods *et al*., 2003) and marine phytoplankton (Toseland *et al*., 2013). With cold hardening in terrestrial plants there are increases in RNA content, but this is accompanied by a marked decrease in growth rate (e.g. Sarhan & D'Aoust, 1975) that is not a consideration here. We do not dispute that decreased growth rate and increased RNA content per unit dry weight are characteristics of cold hardening terrestrial plants. The difficulty with interpreting data for single cells is that cell volume (Atkinson *et al*., 2003) and cell dry weight (Cook, 1966; Aaronson, 1973) increase with decreasing temperature. With decreasing temperature the amount of RNA g^{-1} dry weight *decreases* in *Ochromonas danica* (Aaronson, 1973), and *Euglena gracilis* strain Z (Cook, 1966), but there is a slight (12%) increase in *E. gracilis* var. *bacillaris* between 15 and 25°C (Cook, 1966). When the RNA content is expressed per cell in *Scenedesmus* sp., there is an increase in RNA content with decreasing temperature, but there is also an increase in cell volume, though the relative increase in RNA and P content between 10 and 15°C is greater than that for cell volume (Rhee & Gotham, 1981). However, at 15°C RNA constitutes 23% of total P, but at 10°C, only 13%. In *Fragilariopsis cylindrus* there is a strong negative relationship between temperature (−2, 4 and 10°C) and RNA content per cell (Toseland *et al*., 2013). The optimum growth temperature for *F. cylindrus* is about 4°C, and no growth occurs at 10°C (Lacour *et al*., 2017), therefore it is not surprising that there is less RNA per cell at 10°C. At 0°C, the growth rate of *F. cylindrus* is lower than at 4°C (Zhu *et al*., 2016). RNA increases by *c*. 75% between −2 and 4°C (Toseland *et al*., 2013), and cell volume increases by *c*. 90% between 0 and 4°C and there are similar increases in C, N and P per cell (Zhu *et al*., 2016).

Role of P-constituents other than RNA

Though there are numerous P-containing compounds in a cell, there are only five categories of P-containing compound that are quantitatively significant. The greater requirement for P in fast-growing organisms is either because it requires more of each category of P-containing compound (i.e. the relative proportion of each category remains constant), or one or

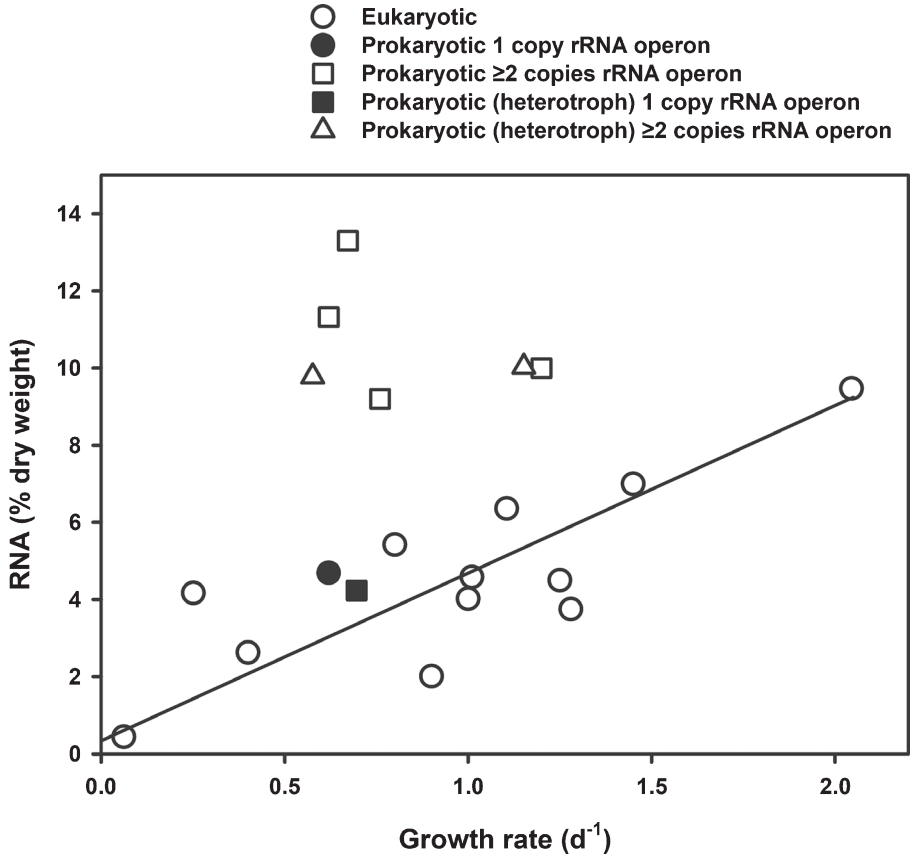

Fig. 3 Relationship between maximum growth rate (d^{-1}) and RNA content (% dry weight) of eukaryotic photosynthetic organisms and prokaryotic photosynthetic organisms and heterotrophs. The reduced major axis regression equation and coefficient of determination for the relationship between maximum growth rate and eukaryote RNA content are as follows: $y = 0.34 + 4.35x$; $r^2 = 0.66$, P (slope = 0) < 0.001, $n = 12$. Data were obtained from the following sources: for eukaryotic photosynthetic organisms, Robson *et al*. (1959), Nyholm (1977), Cook (1981), Kato & Asakura (1981), Laws *et al*. (1983), Bajaj (1970), Fidalgo *et al*. (1995), and Mahboob *et al*. (2012); for prokaryotic photosynthetic organisms with one copy of the rRNA operon, Casey *et al*. (2016); for prokaryotic photosynthetic organisms with two or more copies of the rRNA operon, Kramer & Morris (1990), Fontes *et al*. (1992), Vargas *et al*. (1998), and Li *et al*. (2014); for prokaryotic (heterotrophic) organisms with one copy of the rRNA operon, Cox (2004); for prokaryotic (heterotrophic) organisms with two or more copies of the rRNA operon, Cox (2004). The data for terrestrial plants consisted only of values for suspension or callus/tissue cultures. The data for prokaryotic (heterotrophic) organisms with two or more copies of the rRNA operon are for *Streptomyces coelicolor* growing at 8 and 16% of maximum growth rate (7.2 d^{-1}) so that growth rates were comparable to the other prokaryotes.

more categories of P-containing compound become proportionally more important (and others less so) as μ_{max} increases. The data reported here and in Raven (2013a, his Table 2) support the former hypothesis. Is this because, as μ_{max} increases, more ribosomes are needed and more of the other four categories of P-containing compounds are required to drive RNA synthesis and/or ribosome functioning?

Polyphosphate/phosphate/phytate

Polyphosphates are linear polymers of up to hundreds of phosphate molecules and can be the major P-containing compound in some photosynthetic organisms. It is generally considered to be a form of P storage (John & Flynn, 2000; Martin *et al*., 2014), with the advantage over phosphate being that its accumulation has no osmotic effect (Raven & Knoll, 2010). Polyphosphate may also serve as an energy source (e.g. Kornberg *et al*., 1999), but, at best, this would only provide a very short-term source of energy and ignores the cost of synthesizing polyphosphate (Raven & Knoll, 2010; Lavoie *et al*., 2016). In the aquatic angiosperm *Spirodela oligorrhiza*, 71% of total P is present as phosphate (Bieleski, 1968). Phytate (inositol hexakisphosphate) is the principal form of P storage in tubers, fruits, and seeds of terrestrial plants (Veneklaas *et al*., 2012; Frank, 2013; Lorenzo-Orts *et al*., 2020) and is the major form of P in some plants (Frank, 2013). In the root cortex of *Trifolium subterraneum*, P is accumulated in globular structures together with, in quantitative order, potassium, magnesium, sulphur, sodium and calcium (Ryan *et al*., 2019).

A relationship between polyphosphate concentrations and growth rate has been suggested for *Scenedesmus* sp. (Rhee, 1973) and *Saccharomyces cerevisiae* (Trilisenko & Kulakovskaya, 2014). A barley mutant with decreased (by > 90%) concentrations of seed phytate displays decreased yield even in irrigated fields, but even moderate decreases (33–70%) in phytate in mutant seeds cause decreased yields in nonirrigated fields (Raboy, 2007).

Polyphosphate can act as a chaperone in protein folding in bacteria (Gray *et al*., 2014). The only bacterial chaperone known

to bind to the ribosome is trigger factor, with chaperones such as the ATP-dependent chaperone DnaK binding to released polypeptides (Kramer *et al.*, 2019). In bacteria it is the ATP-dependent chaperones such as DnaK that can be replaced by polyphosphate (Gray *et al.*, 2014). Polyphosphate can bind to ribosomes in *Escherichia coli* both *in vitro* and *in vivo*, and stabilizes polysomes (McInerney *et al.*, 2006). RNA associates with polyphosphate in *Anabaena variabilis* and *Chlorella pyrenoidosa*, with 1 mol of nucleotide being associated with 7 mol polyphosphate-P (Correll & Tolbert, 1962). It is currently unknown whether polyphosphate can act as a chaperone in eukaryotes, but it can bind to ribosomes.

Of particular interest is the role of lysine polyphosphorylation in ribosome biosynthesis in yeast and humans (Bentley-DeSousa *et al.*, 2018; Lorenzo-Orts *et al.*, 2020; McCarthy *et al.*, 2020). In yeast *c.* 7% of proteins have one or more PASK-like motifs (stretches of 20 amino acids with up to at least 15 glutamate, aspartate and serine residues and at least one lysine residue), and the 17 known polyphosphorylated proteins are preferentially localized to the nucleolus. Mutants lacking Vtc4 polyphosphate polymerase are defective in 80S monosome and polysome assembly that compromises ribosome synthesis (Bentley-DeSousa *et al.*, 2018). Two polyphosphorylated proteins in yeast are the histone chaperones Fpr3 and Fpr4 (Bentley-DeSousa *et al.*, 2018). They work cooperatively to regulate genes involved in polyphosphate metabolism and ribosome synthesis, and mutants lacking genes for both chaperones have a genome instability phenotype at rDNA (Savic *et al.*, 2019). The authors suggest that Fpr3 and Fpr4 may act as master regulators of ribosome biosynthesis. What is not known is whether lysine polyphosphorylation occurs in photosynthetic organisms or, even in yeast, the proportion of total polyphosphate that is involved.

Phospholipids

Phospholipids can be replaced by sulpholipids and galactolipids under conditions of P limitation (Andersson *et al.*, 2005; Van Mooy *et al.*, 2009; Raven, 2013b). Because it is assumed that there are no resource limitations during μ_{max}, such substitutions are ignored. However, there is currently no explanation for the apparent superiority of phospholipids under conditions of P sufficiency (Raven, 2013a). In algae and higher plants most extra-chloroplastic membranes are normally dominated by phospholipids (Jouhet *et al.*, 2004; Andersson *et al.*, 2005; Dörmann, 2005; Khozin-Goldberg, 2016), and this may explain the higher phospholipid content of roots compared to leaves (Siebers *et al.*, 2015). Consequently, there will be always be a greater demand for phospholipids in eukaryotic than prokaryotic photosynthetic organisms, because the latter do not contain nuclei, vacuoles, endoplasmic reticulum (ER), Golgi apparatus or mitochondria. Though bacteria do contain organelles, those that are found in cyanobacteria are carboxysomes, which are enclosed in a protein shell and (except in *Gloeobacter*) thylakoids with organelles that are delimited by lipoprotein membranes with very little phospholipid (Greening & Lithgow, 2020). Lipoprotein membranes with phospholipids as the dominant lipid (chromatophores, anammoxosomes and magnetosomes), with the possible exception of lipid bodies, are not found in cyanobacteria (Greening & Lithgow, 2020). This presumably explains the significant difference between the proportion of P present in phospholipids in prokaryotic and eukaryotic photosynthetic organisms.

Phospholipids are important constituents of the nuclear membrane, tonoplast, ER, Golgi apparatus and mitochondrial inner and outer membranes, as well as the plasma membrane, in plants (Dörmann, 2005). In Arabidopsis 31% of the genome codes for membrane proteins (Stevens & Arkin, 2000) that will be synthesized on ribosomes attached to the ER (rough ER) (Staehelin, 1997; Sadowski *et al.*, 2008). Among eukaryotes, the proportion of the genome that codes for membrane proteins is relatively constant at *c.* 30% (Stevens & Arkin, 2000) despite marked differences in μ_{max} suggesting that the proportion of total ribosomes attached to the ER is also relatively constant. The data of Stevens & Arkin (2000) are derived from the predicted hydrophobic α-helical membrane proteins coded in the sequenced genome. For the human genome, only 11% had been sequenced, with 29.7% of the total genome predicted to contain genes coding for membrane proteins. Subsequently, of 21 416 annotated genes in the human genome, about 26% corresponded to membrane proteins (Fagerberg *et al.*, 2010), and in a mapping study of 12 000 human proteins 27% were membrane proteins (Thul *et al.*, 2017). The predictions of Stevens & Arkin (2000) appear to be remarkably robust. Consequently, as the total amount of RNA g^{-1} dry weight increases with increasing μ_{max}, the total number of ribosomes and the number of ribosomes attached to the ER should also increase.

Phosphate esters and anhydrides

Phosphate esters and anhydrides are involved in a variety of both biosynthetic and catabolic pathways. P-esters and anhydrides make up 5.6% of total P in *S. oligorrhiza* (Bieleski, 1968). The major P-esters and anhydrides (in terms of P content) in *S. oligorrhiza* are glucose-6-phosphate, ATP and phosphoglycerate (Bieleski, 1968), and in *Chlamydomonas reinhardtii* are ATP, 3-phosphoglycerate and glucose-6-phosphate (Mettler *et al.*, 2014). It is likely that the value of 3% of total P for *Prochlorococcus* (Casey *et al.*, 2016) is an underestimate as it does not include the three major compounds found in *S. oligorrhiza* or *C. reinhardtii*. Conversely, the value of 17.7% for *Synechococcus elongatus* (Grillo & Gibson, 1979) may be an overestimate as the 5% cold trichloroacetic acid extract could also include orthophosphate and low molecular weight polyphosphate (Herbert *et al.*, 1971; Thompson *et al.*, 1994).

There are currently insufficient data with which to draw any conclusions regarding the amount of P-esters and anhydrides g^{-1} dry weight, but the available evidence suggests that changes in their amount with increasing μ_{max} is likely to be minor, mainly because of adverse osmotic effects (Park *et al.*, 2016; Raven, 2018).

DNA

DNA accounts for a small proportion of dry weight (mean value of 0.5% dry weight) and total P (9%), but the amount of DNA g^{-1} dry weight is greater in fast-growing microalgae than in slow-growing macroalgae and an angiosperm. There is a positive relationship between rDNA copy number and genome size in plants (Prokopowich *et al.*, 2003), suggesting that there are more rDNA copies in faster growing microalgae and providing a potential link between DNA and RNA content.

However, there is a negative relationship between DNA content (though not expressed g^{-1} dry weight) and growth rate (Bennett, 1972; Shuter *et al.*, 1983; Gregory, 2001; Hessen *et al.*, 2010; Sharpe *et al.*, 2012; Šimová & Herben, 2012; Raven *et al.*, 2019a,b). This may (Cavalier-Smith, 1978) or may not (Shuter *et al.*, 1983) affect the rate of RNA transport through the nuclear pores in eukaryotes. There is no effect of genome size on growth rate in prokaryotes (Vieira-Silva *et al.*, 2010).

There is a strong positive relationship between cell length and cell volume and the rRNA gene copy number in marine microalgae (Zhu *et al.*, 2005; Godhe *et al.*, 2008; Raven *et al.*, 2019a) that would translate into a relatively low rRNA gene copy number in fast growing organisms. The extra copies in yeast are thought to protect the cells against DNA damage from mutagens such as UV. This became essential with the evolution of larger eukaryotic cells that required more rDNA transcription, which would be toxic unless they maintained more rDNA copies (Ide *et al.*, 2010), though small, fast-growing cells are more at risk from UV damage than large cells (Raven, 1991; Finkel *et al.*, 2010).

RNA

In eukaryotic or prokaryotic photosynthetic organisms, the proportion of total P which is RNA is surprisingly constant, irrespective of growth rate. In eukaryotes, the RNA content constitutes 20% of total P in *Parthenocissus tricuspidate*, which grows at 0.06 d^{-1} (Robson *et al.*, 1959), 27% in *Chlorella vulgaris*, which grows at 2.05 d^{-1} (Nyholm, 1977) and is 17–32% in eight species of macroalgae (Young, 1964). The linear relationship between μ_{max} and RNA in eukaryotes suggests that RNA makes up a relatively constant 25% of total P. By contrast, in cyanobacteria RNA is 57% of total P in *P. marinus* growing at 0.62 d^{-1} (Casey *et al.*, 2016) and 54% in *S. elongatus* growing at 3.36 d^{-1} (Grillo & Gibson, 1979). RNA remains a remarkably constant proportion of total P in cyanobacteria also, but closer to 50% of total P rather than the 25% in eukaryotic photosynthetic organisms.

Bacteria have from 1 to 17 copies of the rRNA operon, which consists of the three genes that encode 16S, 23S and 5S rRNA together with internal transcribed spacer regions that contain tRNA (Espejo & Plaza, 2018). The advantage of possessing more than one copy is that it allows rapid responses to increased resource availability (Condon *et al.*, 1995; Klappenbach *et al.*, 2000), but bacteria adapted to low-nutrient environments tend to be slow-growing and have a low number of rRNA operon copies (Fegatella *et al.*, 1998; Klappenbach *et al.*, 2000). There is a positive relationship between rRNA operon copy number and growth rate in bacteria and Archaea, though there is a large range of growth rates within each rRNA operon copy number up to a total of five (Vieira-Silva & Rocha, 2010). Of particular interest is a comparison of *Bacillus subtilis* with one to ten copies (Yano *et al.*, 2013). The largest difference in the phenotypes of this bacterium occurs in the transition from one to two copies (Yano *et al.*, 2013), and mutants with a single copy have lower numbers of ribosomes (20–35%) than the wild-type (Nanamiya *et al.*, 2010). Consistent with this observation, a mutant of *E. coli* with only one copy of the rRNA operon has 56% of the rRNA found in a strain with no deletions, but the decrease in total RNA is relatively minor because of the presence of increased levels of tRNA in the mutant (Asai *et al.*, 1999); the growth rate of the mutant with only one copy is *c.* 50% that of the wild-type. The increased levels of tRNA are due to the increase in the tRNA : ribosome ratio that occurs with decreased growth rate in *E. coli* (Dong *et al.*, 1996). Though there are no published values for μ_{max}, all 62 genomes of *Rickettsia* investigated have a single operon (The Ribosomal RNA Database) and RNA is 3 to 5.5% of dry weight in *Rickettsia burneti* (Smith & Stoker, 1951), which is similar to the values for *P. marinus* subsp. *pastoris* str. CMP1986 (also known as Med4) and *M. bovis*, which also have a single copy of the rRNA operon.

In contrast to cyanobacteria, there was a strong positive relationship between μ_{max} and organism RNA content in eukaryotes, as predicted by the growth rate hypothesis. There is no doubting the central importance of RNA, but where is the other 75% of total P for eukaryotic photosynthetic organisms – and 50% for cyanobacteria and heterotrophs (Elser *et al.*, 2003) – located, and what is its contribution to growth rate (Moreno & Martiny, 2018)?

Paucity of data

There are two major reasons for the limited data. The first is the use of dry weight as a universal measure of biomass across all photosynthetic organisms. The second is the time-consuming and unrewarding nature of compiling inventories of biochemical composition. While the use of dry weight as a measure of biomass is relatively common in macroalgae and terrestrial plants, it is much less so in microalgae and cyanobacteria. A major reason for the latter is the inconvenience of measuring dry weight due to salts in residual medium, on filters or in centrifuge tubes compromising the final dry weight. However, this is easily overcome by washing the cells with isotonic ammonium formate, which is volatile and is lost when the cells are dried using heat. Good inventories are available for well-studied organisms such as *E. coli*, but sadly the incentive for their construction is very limited. However, where they are available (e.g. Park *et al.*, 2016) their considerable value is obvious.

Conclusion

Two of the three key predictions of the (maximum) growth rate hypothesis hold for eukaryotic photosynthetic organisms. However, RNA does not account for most of the P and the proportions of the different classes of compounds that contain P stay relatively constant. There is clear evidence that as μ_{max} increases, the amount of

RNA g^{-1} dry weight increases, and there is some evidence that more phospholipids are required and, to a lesser extent, polyphosphate and DNA (expressed g^{-1} dry weight), but it is unlikely that the amounts of phosphate esters and anhydrides increase. Whether the classes of compounds other than RNA are involved, directly or indirectly, in RNA biosynthesis and ribosome function (as suggested here) and/or they have other important roles in cell metabolism that increase with increasing μ_{max} remains to be elucidated.

Acknowledgements

We thank Adam Martiny and an anonymous reviewer for their constructive comments on an earlier version of the manuscript. JAR thanks Hans Lambers and Mark Westoby for their continued support and encouragement.

Author contributions

TAVR collected and analysed data; TAVR and JAR wrote the manuscript.

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Data availability

The data obtained for this study are available at https://www.doi.org/10.6084/m9.figshare.13378145.

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